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Paecilomyces wawuensis, a new species isolated from soil in China

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ABSTRACT — A new species, *Paecilomyces wawuensis*, isolated from soil samples of the Wawu Mountain, Sichuan Province, China, is described and illustrated. It is characterized by phylogenetic analysis of ITS rDNA and β-tubulin gene sequences, white obverse and sandy beige reverse colonies that grow slowly on Czapek agar, cylindrical phialides with long, thin necks, and ellipsoidal-ovate, spinulose conidia.

KEY WORDS — taxonomy, fungi, hyphomycete

Introduction

The hyphomycete genus *Paecilomyces* was introduced by Bainier (1907) to accommodate *Paecilomyces variotii* Bainier, a species growing in the air, soil, and compost and on wood. It was characterized by verticillate conidiophores with divergent whorls of phialides, which have a cylindrical or inflated base tapering into a long and distinct neck. The conidia are typically hyaline, one-celled, smooth-walled, and produced in basipetal chains. The genus was revised by Brown & Smith (1957) and further modified by Samson (1974), who accepted 31 species. Some species of *Paecilomyces* were later found to represent anamorphs of *Byssochlamys* Westling, *Talaromyces* C.R. Benj., *Thermoascus* Miehe, *Cordyceps* Fr., and *Torrubiella* Boud. (Stolk & Samson 1972, Samson 1974).

In *Paecilomyces,* most species can be separated from each other with colonies, conidiogenous cells, conidia, and phialides. Han (2007) accepted only 64 of the 125 published *Paecilomyces* species. According to Index Fungorum (www.indexfungorum.org), over 140 epithets were assigned to *Paecilomyces;* many of them, however, are known to be taxonomic synonyms or dubious taxa.

A total of 39 species in *Paecilomyces* have been reported in China, including 18 entomogenous species and 21 species from soil (Liang et al. 2005a,b, 2006; Han et al. 2005a,b,c; Li et al. 2006).

Based on the records of *Paecilomyces* and the distinctive molecular and morphological characteristics, the fungus isolated from soil samples of the Wawu Mountain, Sichuan Province, China, is described as a new species of *Paecilomyces*.

Materials & methods

Collection and strain isolation

Strain GZU-BCECWS15 was isolated from a soil sample collected from the Wawu Mountain, Sichuan Province, China, in July, 2008. Two grams of soil were added to a flask containing 20 ml sterilized water and glass beads. The soil suspension was diluted to a concentration of 10^{-1}–10^{-3} after shaking for about 10 min. A 1 ml soil suspension (concentration 10^{-3}) was mixed with Martin medium in sterilized 9 cm diam Petri dish and incubated at 25°C for 5 days. The strain was purified by transplanting to Martin's slants.

Morphological identification of strain

The strain studied was transplanted onto Czapek agar, potato dextrose agar (PDA), and Sabouraud agar. After incubating at 25°C for 14 days, morphological identification of the strain was carried out based on colony characters, conidiogenous structure and other biological features (Brown & Smith 1957, Samson 1974).

The generic names *Paecilomyces* and *Penicillium* are abbreviated as "*P.*" and "*Pen.*" respectively. The holotype GZU-BCECWS15 of *P. wawuensis* was deposited in the Engineering and Research Center for Southwest Bio-Pharmaceutical Resources of National Education Ministry of China, Guizhou University, PR China.

Table 1. ITS rDNA sequences from GenBank used in the phylogenetic analysis

Name	GenBank No.	Name	GenBank No.
P. antarcticus	AJ879113	*P. gunnii*	AJ309339
P. carneus	AY624171	*P. marquandii*	AY624193
P. carneus	AB103179	*P. niphetodes*	AY624192
P. carneus	DQ888728	*P. penicillatus*	AY624194
P. carneus	DQ914684	*P. viridis*	AY624197
P. gunnii	AJ309343	*P. wawuensis*	GU453921
P. gunnii	GY453920	*Pen. expansum*	AF404655

Table 2. β-tubulin gene sequences from GenBank used in the phylogenetic analysis

Name	GenBank No.	Name	GenBank No.
P. carneus	AY624209	*P. viridis*	AY624235
P. carneus	AY624210	*P. wawuensis*	HM480496
P. gunnii	HM480497	*Pen. expansum*	AF003248

DNA sequencing & phylogenetic analysis

Genomic DNA was extracted from fungal mycelia directly collected from the plates following the CTAB isolation protocol of Oscar et al. (1999). Template DNA (30–40 ng) was amplified in a 50 µl PCR reaction mixture consisting of 10 mM KCl, 10 mM $(NH_4)_2SO_4$, 20 mM Tris-HCl (pH 8.8), 6 mM $MgCl_2$, and 500 µM each of dATP, dCTP, dGTP, and dTTP, with 60 pmols ITS4 & ITS5 primers (White et al. 1990) for ITS rDNA or T1&T22 primers (O'Donnell & Cigelnik 1997) for β-tubulin gene, and 2 units TaqDNA polymerase (TianGen, China). The reaction was set up as follows: initial denaturation at 94°C for 5 min, followed by 30 cycles of denaturation at 94°C for 1 min, annealing at 51°C for 1 min, extension at 72°C for 1 min and final extension at 72°C for 10 min in Gene Amp PCR system 9700 (Gene Amp, U S). The temperature of annealing for β-tubulin gene (TUB2) was 54°C.

The PCR products were purified by using a UNIQ-10 PCR Purification Kit (Sangon Biotech, China). The purified PCR products were sequenced using the ABI Prism 377 DNA Sequencer (Perkin-Elmer, Norwalk, Connecticut) with ITS4 or ITS5 and T1 or T22. The DNA sequences of ITS rDNA and β-tubulin gene of the fungus have been submitted to GenBank.

Nucleotide sequences of ITS rDNA and β-tubulin gene of the isolate GZU-BCECWS15 and their allies as well as the outgroups retrieved from GenBank were assembled using Tex-Edit Plus (Bender, TomBB@aol.com) respectively. The alignment of the sequence files was conducted using the CLUSTAL W software (Thompson et al. 1994). Phylogenetic analyses were performed with PAUP version 4.0b10 (Swofford 2004). The most parsimonious trees (MPT) were determined from the data sets using the heuristic search options with 1000 random sequence input orders with MULPARS on and TBR branch swapping for the exact solution. The unconstrained topologies of the equally parsimonious trees were compared using the Kishino-Hasegawa test of PAUP. The best topology was selected as the most parsimonious tree topology. Parsimony bootstrap with 1000 replicates in PAUP was applied to the tree to evaluate the stability. Other measures including tree length, consistency, retention, rescaled consistency and homoplasy indexes (TL, CI, RI, RC and HI) were also calculated.

Taxonomy

Paecilomyces wawuensis Jin He, J.C. Kang & B.X. Lei, **sp. nov.** FIG. 1

MycoBank MB 518791

On agaro Czapekii, coloniae 25–26 mm diam in 14 diebus ad 25°C, albae, floccosae, margine regularis, reversum vinacea. Hyphae hyalinae, septatis, levibus, 1.3–1.5 µm crassis. Conidiophora breva, simplicia, 16–40 × 1.3–2.0 µm, phialides singulare vel phialidibus 2 ad 4 terminatis, Phialides12–42 × 1.8–4.0 µm, cylindricae, e basi inflata, angustatae collolongo minus quam 0.4 µm. Conidia monocellula, hyalina, verrucosa, ellipsoidea ad ovata vel subglobosa, 4.3–6.9 × 3.3–5.3 µm, facientia divergentes, catenas exsiccates.

Typus: *GZU-BCECWS15 et cultara , isolatus ab soli in solo Wawu, Provinice Sichuan, VII.2008, T.C. Wen; in Guizhou Univ., conservatur.*

Colonies on Czapek agar growing slowly, attaining a diameter of 25–26 mm 14 days at 25°C; white, ridged, dense floccose, thick, with orderly margins, with some irregular radiating furrow and more or less hyaline exudates; reverse

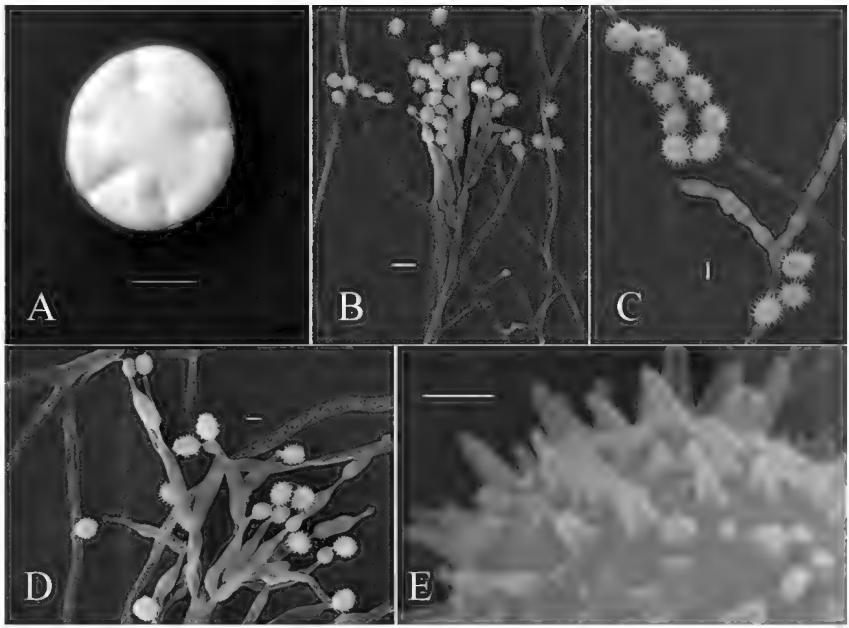

FIG. 1. Photographs of colony and conidiogenous structure of *Paecilomyces wawuensis* under scanning electron microscope. **A.** Colony on Czapek agar after 14 days at 25°C. **B.** Conidiogenous structure. **C.** Ellipsoidal conidia in chains. **D.** Phialides of the conidiogenous structure. **E.** Echinula of a conidium. Scale bars: **A** 10 mm, **B** 5 μm, **C–D** 2 μm, **E** 0.5 μm.

sandy beige, appearing soluble wine red pigment. Vegetative hyphae septate, hyaline, smooth-walled, 1.3–1.5 μm wide. Conidiophores mononematous erect or absent, hyaline, smooth-walled, uneven length, 16–40 × 1.3–2.0 μm, with single phialide or whorls of 2 to 4 phialides, or phialides growing from hyphae directly. Phialides 12–42 × 1.8–4.0 μm, consisting of a cylindrical, somewhat inflated base, tapering into a long and thin neck, somewhat incurved, 6.0–6.7 μm long and less than 0.6 μm wide. Conidia one-celled, hyaline, roughened-walled with spinulose, most ellipsoidal or ovate to subglobose, 4.3–6.9 × 3.3–5.3 μm, forming divergent, dry and basipetal chains. Chlamydospores present, produced singly or in short chains, thick-walled, roughened, globose to subglobose, 7.5–8.5 μm in diameter.

COLONIES ON PDA at 25°C within 14 days, usually growing more rapidly than on Czapek agar, 27mm, consisting of a pink mycelium, with some irregular radiating furrow and more or less hyaline exudates. Reverse saddle brown, with distinct radiating furrow and present wine red pigment diffusion.

COLONIES ON SABOURAUD AGAR 25 mm at 25°C within 14 days, consisting of white mycelium, with some irregular radiating furrow. Reverse light brown, with distinct radiating furrow.

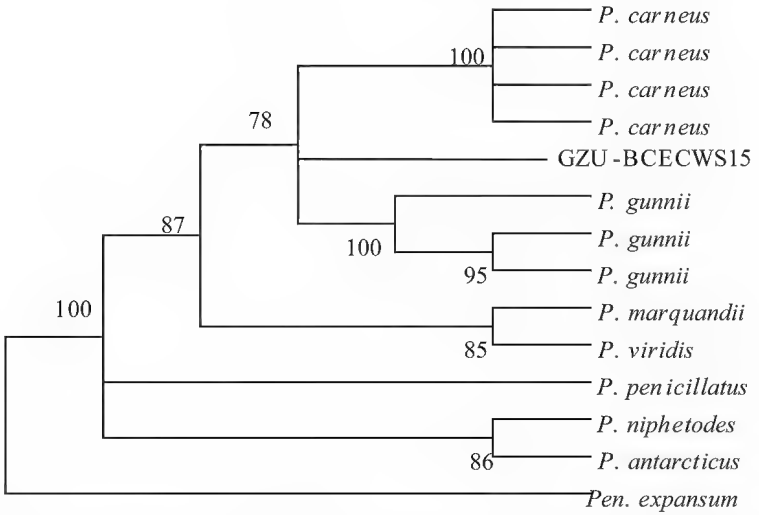

Fig. 2. The gene tree was constructed using the MP method and based on phylogenetic analysis of the nucleotide sequences of ITS1-5.8S-ITS2 rDNA. It shows the relationships between GZU-BCECWS15 and other *Paecilomyces* species. Bootstraps values (1,000 replicates) are indicated at the nodes (TL 577, CI 0.825, HI 0.175, RI 0.710, RC 0.586).

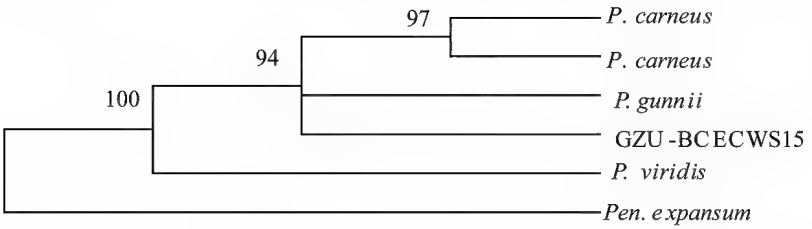

Fig. 3. The gene tree was constructed using the MP method and based on phylogenetic analysis of the β-tubulin gene sequences. It shows the relationships between GZU-BCECWS15 and other *Paecilomyces* species. Bootstraps values (1,000 replicates) are indicated at the nodes (TL 174, CI 0.937, HI 0.063, RI 0.645, RC 0.604).

Phylogenetic analyses

The ITS1-5.8S-ITS2 rDNA of the *P. wawuensis* has been sequenced and compared with that of *P. carneus* (CBS 239.32, GenBank Accession No.: AY624171) and *P. gunnii* (GenBank Accession No.: AJ3093390) in an alignment. In comparison with *P. carneus*, *P. wawuensis* exhibited 31 transitions/transversions and 36 deletion and 6 insertion substitutions. Meanwhile there were 60 positional transitions/transversions and 57 insertion/deletion substitutions in ITS1-5.8S-ITS2 rDNA between *P. gunnii* and *P. wawuensis*.

At the same time, the alignment of β-tubulin gene sequences from *P. gunnii, P. carneus,* and *P. wawuensis* showed that *P. wawuensis* had 1 insertion substitution, 6 deletion substitutions and 12 transitions/transversions both to *P. gunnii* and *P. carneus*.

Both the most parsimonious trees (MPT) inferred from the ITS rDNA (Fig. 2) and the β-tubulin gene sequences data (Fig. 3) showed that the strain GZU-BCECWS15 stands parallel to the others, although it is clustered with *P. gunnii* and *P. carneus* in a subclade.

Discussion

In the genus *Paecilomyces*, rare species have been found to have echinulate conidia. The previously accepted species with echinulate conidia were *P. carneus* Brown & Smith (Samson 1974), *P. gunnii* Z.Q. Liang (Liang 1985), and *P. curticatenatus* Z.Q. Liang & Y.F. Han (Han et al. 2007). Liang et al. (2009) recombined *P. curticatenatus* in a new genus, *Taifanglania*, characterised by solitary phialides; this character separates *P. curticatenatus* from *P. wawuensis*. The new species *P. wawuensis* can be separated from *P. carneus* and *P. gunnii* by the sandy beige reverse of the colony on Czapek agar and the large ellipsoidal-ovate conidia (Table 3). *Paecilomyces wawuensis* can be distinguished from the other species in having a white/ sandy beige colony on Czapek agar and echinulate conidia.

Table 3. A comparison between *Paecilomyces wawuensis* and its related species

Species	Colony		Phialides	Conidia	Chlamydospores
	obverse	reverse			
P. carneus	white	olive drab	cylindrical	subglobose to ellipsoidal 3 4 × 2 2.5 µm	absent
P. gunnii	grey	brown	cylindrical	subellipsoidal or subovate 1.6 4.8 × 1.2 3.5 µm	present
P. wawuensis	white	sandy beige	cylindrical/ ampulliform	ellipsoidal or ovate to subglobose 4.3 6.9 × 3.3 5.3 µm	present

Meanwhile the dominant substitutions and transitions/transversions in the alignments of ITS1-5.8S-ITS2 rDNA and β-tubulin gene sequences of *P. carneus*, *P. gunnii,* and *P. wawuensis* differentiate the new species from the others.

In conclusion, *Paecilomyces wawuensis* can be distinguished from the other species in the genus by the following morphological characters: 1) colonies on Czapek agar grow slowly and the obverse is white while the reverse is sandy beige; 2) phialides consist of a cylindrical, sometimes inflated base, tapering into a long, thin, and sometimes incurved neck; and 3) conidia are ellipsoidal or ovate to subglobose, large and roughened-walled with spinules.

Both phylogenetic trees (Figs. 2, 3) based on ITS rDNA and β-tubulin gene sequences show that the strain GZU-BCECWS15 stands parallel to *P. carneus*

and *P. gunnii* in a sub-clade, although all of them possess roughened-walled conidia with spinules. Both molecular and morphological evidence supports *P. wawuensis* (GZU-BCECWS15) as a new species.

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